

## SUPPLEMENT

# $\alpha$ -Catenin unfurls upon binding to vinculin

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### Supplementary Figure Legends

**Supplementary Fig. S1. Stereo view of the final Vh1:VBD electron density map.** The  $2F_o - F_c$  map is depicted in cyan at a contouring level of  $0.5\sigma$ . Vinculin and  $\alpha$ -catenin carbon atoms are shown in yellow and in white, respectively; nitrogen atoms are in blue, oxygen in red, and sulfur in dark yellow.  $\alpha$ -Catenin residues 355 through 361 connecting the last two  $\alpha$ -catenin  $\alpha$ -helices are labeled. Importantly, the structure reveals a clear structural definition in the connector region.

### Supplementary Fig. S2. Novel features of the Vh1: $\alpha$ -catenin complex.

A. Superposition of five talin-VBS (dark grey) bound structures in complex with Vh1 (light grey) onto our  $\alpha$ -catenin VBD (blue, magenta, and cyan) bound to Vh1 (yellow): PDB entry 1xwj (talin-VBS3, residues 1945-1969) aligns with *r.m.s.d.* of 0.9 Å for 1,688 atoms; PDB entry 1zw2 (talin residues 2,345-2,369), 0.9 Å for 1,721 atoms; PDB entry 1zw3 (talin residues 1,630-1,652), 1 Å for 1,750 atoms; PDB entry 1u6h (talin-VBS2, residues 849-879), 0.9 Å for 1,727 atoms; and PDB entry 1t01 (talin-VBS1, residues 605-628), 1.2 Å for 1,836 atoms. All of the talin-bound structures represent binding of only a short talin-VBS peptide, while we solved for the first time a VBD in complex with Vh1, which shows extensive and novel interactions and captures the structure of an unfurled vinculin binding partner.

B. Superposition of Vh1 (light grey) in complex with the *Shigella* invasin IpaA VBS2 (dark grey) onto our  $\alpha$ -catenin VBD (blue, magenta, and cyan) bound to Vh1 (yellow) highlights the novel  $\alpha$ -catenin binding site on the C-terminal Vh1 subdomain. The shape correlation statistic derived using the CCP4 program SC is greater for the C-terminal Vh1 subdomain with  $\alpha$ -catenin (0.72) *versus* IpaA (0.65) with a slightly increased contact area ( $\sim 1,313$  Å<sup>2</sup> *versus*  $\sim 1,281$  Å<sup>2</sup>). A symmetry related  $\alpha$ -catenin  $\alpha$ -helix is shown in cyan. Vinculin  $\alpha$ -helices and  $\alpha$ -catenin and IpaA termini are labeled (IpaA termini are underlined and italicized).

**Supplementary Fig. S3. Schematic presentation of the Vh1:VBD interactions.**

$\alpha$ -Catenin residues are boxed in light orange and Vh1 residues engaging in polar or hydrophobic interactions are indicated to the left or right of the respective  $\alpha$ -catenin residue. Residues labeled in blue engage in hydrogen bonds while those in red direct electrostatic interactions.

A.  $\alpha$ -Catenin residues 305-316 reside on the  $\alpha$ -helix bound to the C-terminal Vh1 subdomain, residues 317-319 bind to the subsequent loop region, residues 329-351 interact with an  $\alpha$ -helix of the N-terminal Vh1 subdomain, and Met-352 binds to the following loop region. All four  $\alpha$ -helices ( $\alpha$ 1, residues 7-28;  $\alpha$ 2, 41-64;  $\alpha$ 3, 68-97;  $\alpha$ 4, 102-130) of the N-terminal Vh1 subdomain contribute to binding to the  $\alpha$ -catenin VBS (residues 329-351).

B. Interactions of  $\alpha$ -catenin with symmetry-related Vh1 molecules.

**Supplementary Fig. S4. The  $\alpha$ -catenin binding sites are accessible in full-length vinculin.**

Superposition of the full-length vinculin structure (VH, white; Vt, grey) onto Vh1 (yellow) bound to  $\alpha$ -catenin ( $\alpha$ -helices  $\alpha$ 1 and  $\alpha$ 4 are shown in cyan,  $\alpha$ 2 in blue, and  $\alpha$ 3 in magenta). Vh1  $\alpha$ -helices are labeled  $\alpha$ 1 through  $\alpha$ 7, Vt  $\alpha$ -helices are labeled H1 through H5. Terminal residues are labeled. The  $\alpha$ -catenin binding site is fully accessible in the full-length vinculin structure.

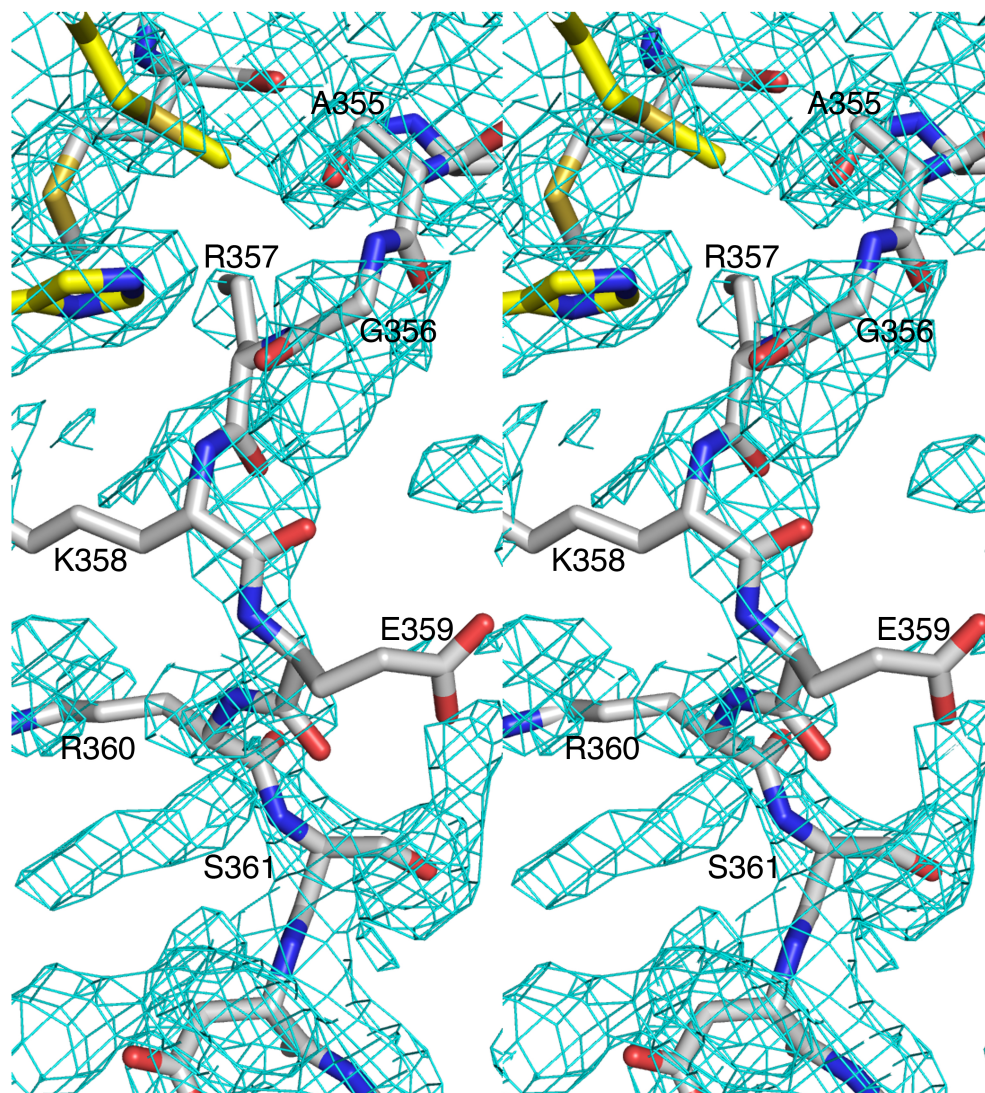
**Supplementary Fig. S5. Contacts of the terminal  $\alpha$ -catenin VBD  $\alpha$ -helices.**

The (A) carboxy- and (B) amino-terminal  $\alpha$ -helices of  $\alpha$ -catenin (shown as ball-and-stick representations) engages in various interactions with symmetry-related Vh1 molecules (yellow, cyan, and magenta, respectively) shown as a cartoon.

A. The interface of the C-terminal  $\alpha$ -catenin VBD  $\alpha$ -helix and the Vh1 molecule shown in cyan forms the dimer of heterodimers shown in Figure 2 and has the largest shape complementarity compared to the interactions of  $\alpha$ -catenin (shown in grey) with the Vh1 molecules shown in magenta or yellow, respectively. Some Vh1  $\alpha$ -helices and  $\alpha$ -catenin residues are labeled.

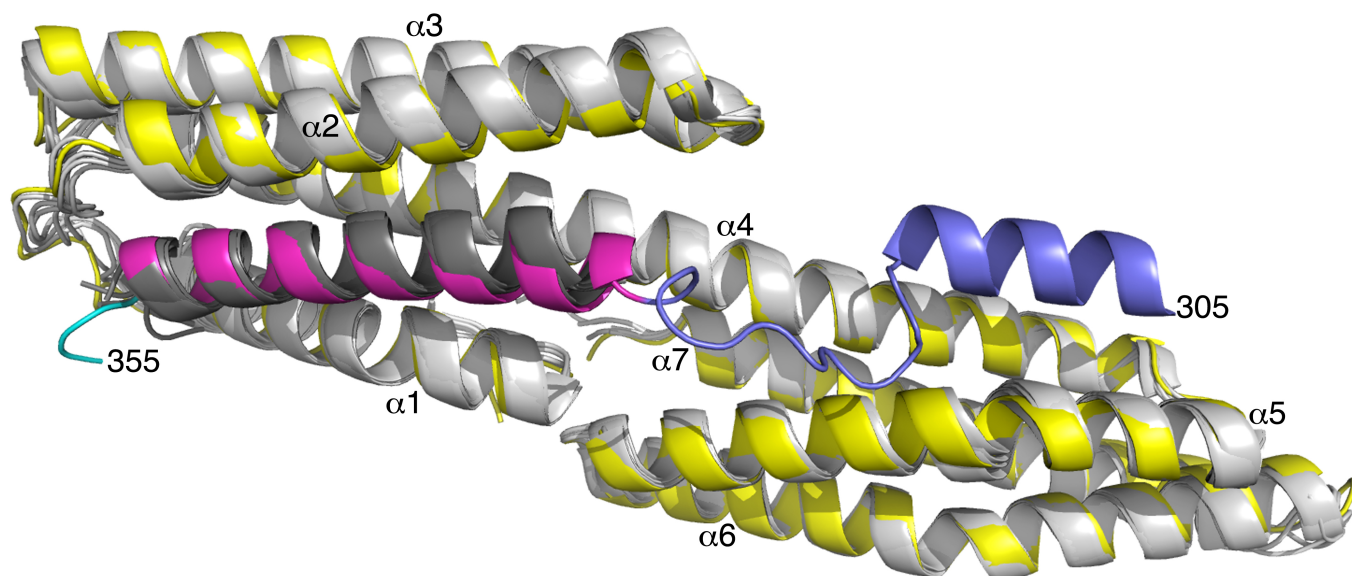
B. Stereo view of the N-terminal  $\alpha$ -catenin VBD  $\alpha$ -helix (shown in green) interaction with symmetry-related Vh1 (shown in magenta). Some  $\alpha$ -catenin residues are labeled.

Supplementary Figure S1

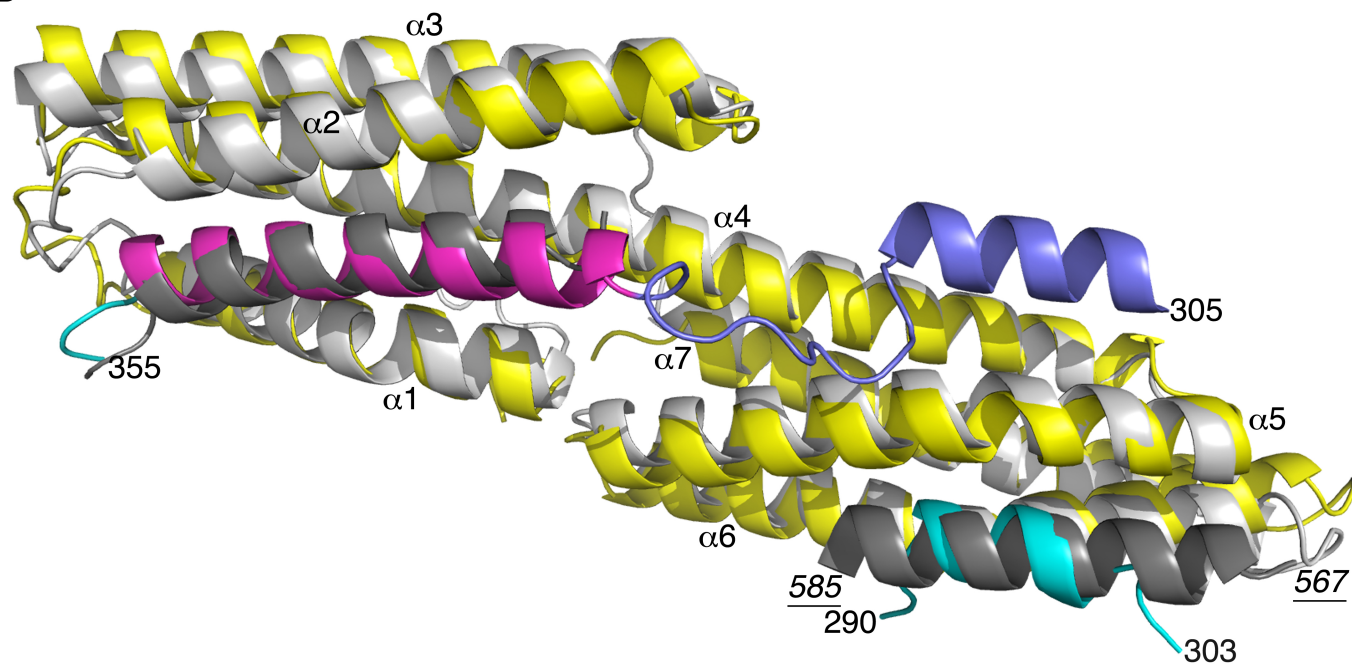


Supplementary Figure S2

*A*



*B*





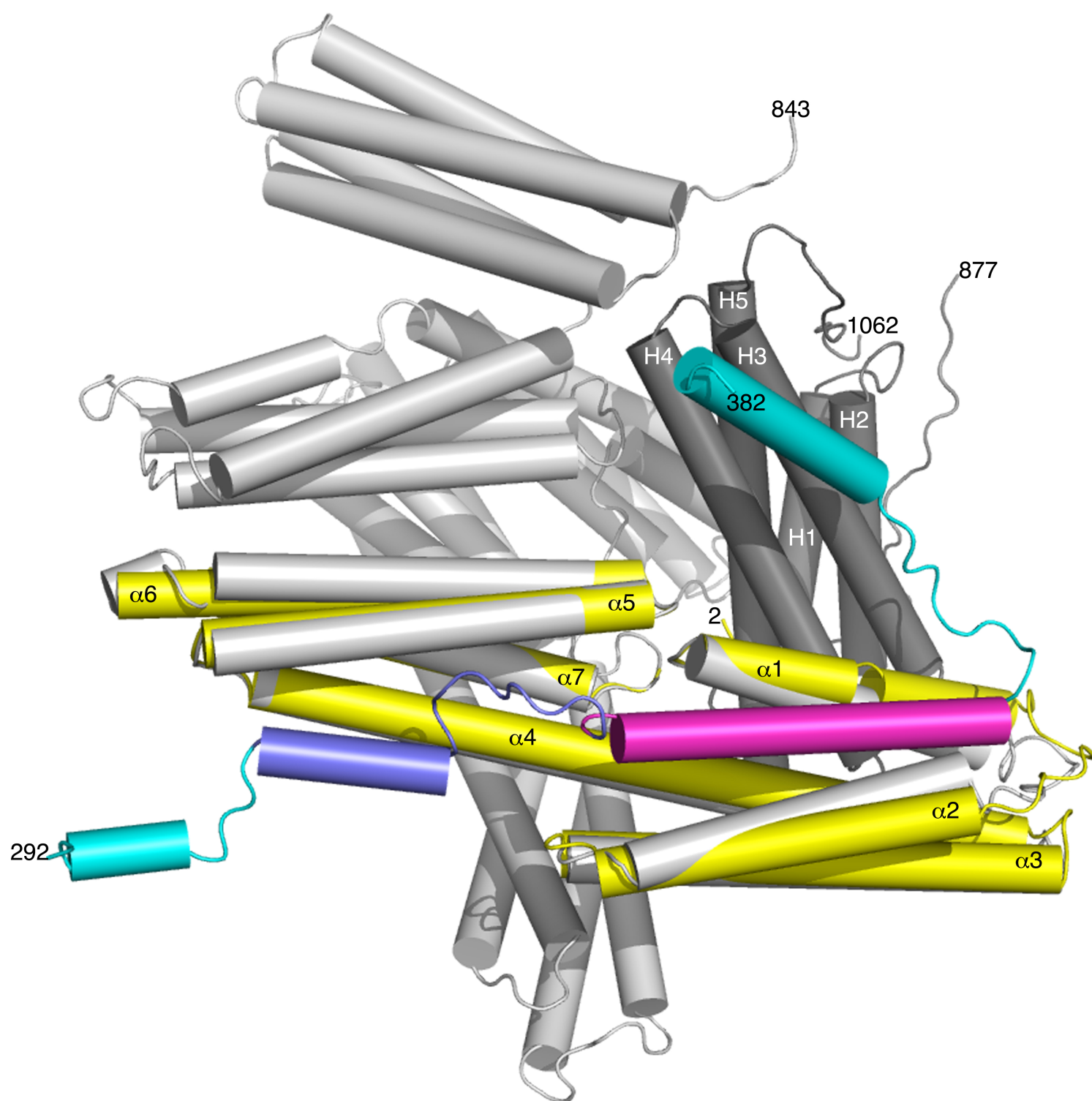
Supplementary Figure S3A

Vh1	$\alpha$ -catenin	Vh1
	Leu305	Val147, Val150, Val151, Asp156
	Glu306	Thr159
	Arg308	Val147
	Leu309	Tyr144, Tyr160
	Ile312	Glu143, Val147
	Ile313	Asn163
Arg136	Gly315	
	Ala316	Gly140
Lys170	Ala317	
	Leu318	Val137, Gly140, Ile141, Gly167
Lys170	Met319	
Thr64, Thr61 Thr8	Arg329	
	Arg330	Phe126
	Arg332	Val157
	Ile333	Val57, Leu122, Phe126
	Val334	Thr8
	Glu336	Leu54, Val57
	Cys337	Ile12, Leu54, Thr119, Leu123
	Asn338	Ile12
	Ala339	Ala50
	Val340	Ala50, Val51, Leu54, Ile115
	Arg341	Val16
	Ala343	Ala46, Val47
	Leu344	Val47, Ser112, Ile115
Gln19	Gln345	Pro15, Val16
	Leu347	Leu40, Pro43, Val44, Val47, Leu88
	Leu348	Gln19, Ile20, Leu23
Gln19	Ser349	
	Glu350	Pro43
His27	Tyr351	Leu23, Ile37, Leu40, Leu108
	Met352	Gln19, Leu23

Supplementary Figure S3B

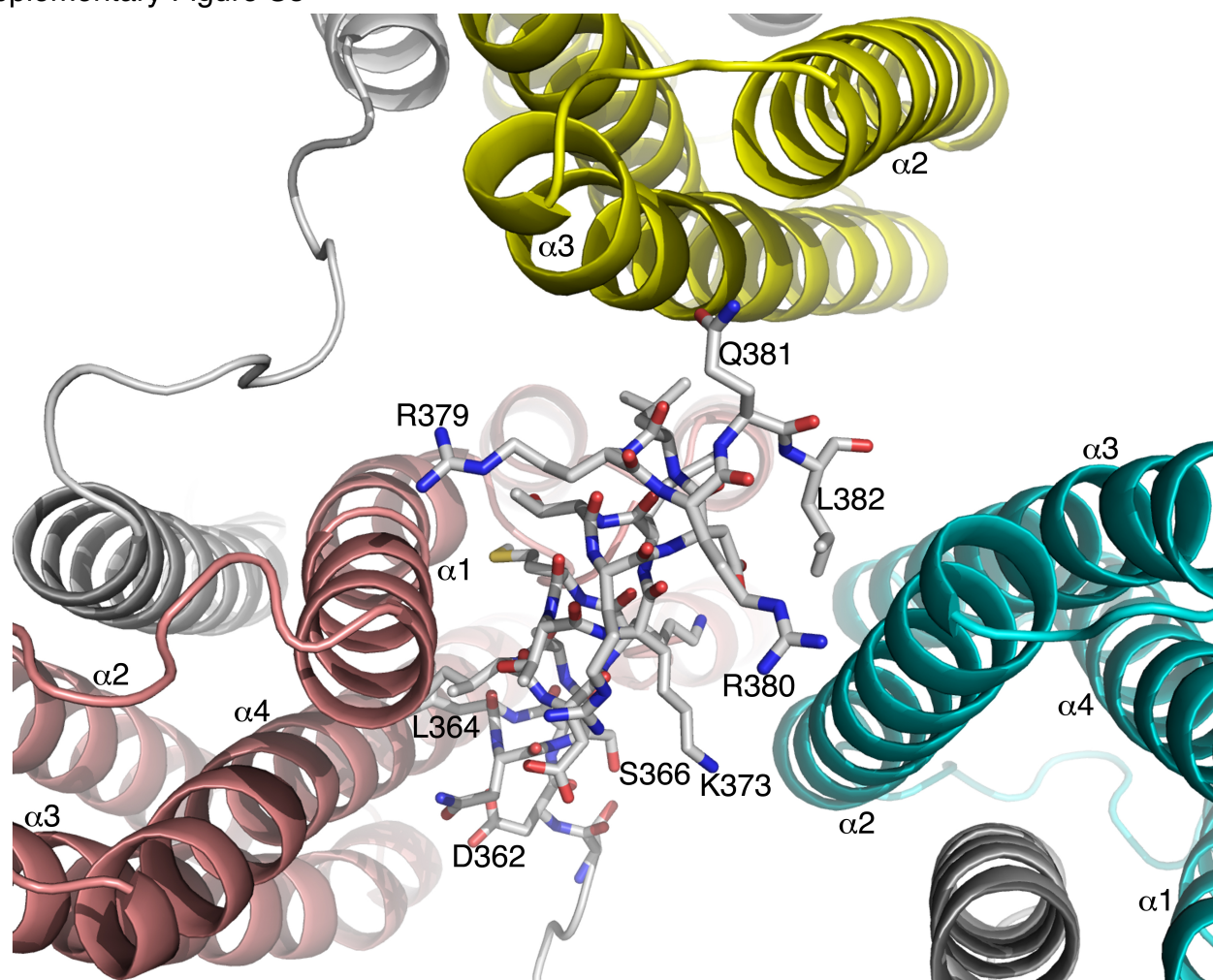
Vh1	$\alpha$ -catenin	Vh1
	Ile290	Asn196, Lys199
	Val291	Pro203
	Pro293	Gly165, Met168, Leu202
Thr169	Leu294	Pro166
	Phe296	Leu202, Pro203, Ile206
Thr161, Gly165	Ser297	Gly165
Glu225	Arg300	Ile206
	Phe301	Leu157, Val158, Thr161, Ile206, Lys210
	Asp362	Ser117
	Leu364	Phe4, Leu16, Ala17, Ser117
Ser117, Arg113	Asn365	
	Ala367	Val3
	Ile368	Ala17, Arg113
Arg113	Asp369	
Ser21	Met371	Ala17, Gln18, Ser21
Ser21	Thr372	Val24, Ile25
Asn53	Lys373	Arg56
	Thr375	Ile25
Glu28	Arg376	
Arg56	Asp377	
	Leu378	Pro75, Ile79
	Arg379	Ile25
Gln63	Arg380	Glu60
Lys71, Gln63	Gln381	
	Leu382	Lys59, Glu60, Gln63

Supplementary Figure S4



Supplementary Figure S5

A



B

